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Human leukocyte interferon produced by *E. coli* is biologically active

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A human leukocyte interferon cDNA was enzymatically synthesized, inserted into the vector pBR322, and cloned in Escherichia coli. *The DNA sequence codes for a 23-amino acid signal peptide followed by an interferon polypeptide of 165 amino acids. An expression plasmid was constructed which permits the synthesis in* E. coli *of* 2.5×10^8 *units of interferon per litre of culture. This LeIF protected squirrel monkeys from lethal encephalomyocarditis virus infection.*

THE interferons (IFNs) are a family of proteins characterized by a potent ability to confer a virus-resistant state in their target cells[1,2]. In addition, IFNs can inhibit cell proliferation and modulate immune response (reviewed in ref. 2). These properties have led to the clinical use of IFN for the treatment of viral infections and malignancies.

The classical, or type I, IFNs consist of at least two distinct gene products which differ antigenically[3,4] and have different target cell specificities[5]. Leukocyte interferon (LeIF) and fibroblast interferon (FIF) are named for the cells from which they are derived. Messenger RNAs for LeIF and FIF have been isolated and translated *in vitro* and in *Xenopus* oocytes[4,6–8].

Both LeIF[9,10] and FIF[11] have been purified to homogeneity; reported molecular weights range from 17,500 to 21,000. The specific activities of these preparations are remarkably high, 2×10^8 to 1×10^9 U per mg protein. Unfortunately the yields from eukaryotic cells have been correspondingly low. Two litres of human blood are required to produce approximately 1 μg of

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purified LeIF[9]. Nevertheless, advances in protein sequencing techniques have permitted the determination of partial amino acid sequences[11–13].

Recombinant DNA technology provides an alternative method of producing large quantities of IFN using bacterial cells. Human FIF cDNA has recently been cloned[14,15] and biologically active mature FIF expressed in *Escherichia coli*[15]. Likewise, a species of human LeIF cDNA has been cloned in *E. coli* and expressed in an apparent precursor form with a signal peptide attached[16]. We describe here the construction of a plasmid directing the high level expression in *E. coli* of a human LeIF which exhibits *in vivo* antiviral activity.

Construction and identification of clones containing LeIF cDNA sequences

Cells from the human myeloblastoid line KG-1 (ref. 17) were collected 5 h after induction with Sendai virus and the 12S sucrose gradient fraction of poly(A) RNA was isolated as described elsewhere[18]. This mRNA had an IFN titre of 8,000 U μg^{-1} in the *Xenopus laevis* oocyte assay[4]. Five μg of mRNA was used to prepare double-stranded cDNA by standard procedures[19,20]. The cDNA was electrophoresed on a 6% polyacrylamide gel and 110 ng of material from 600 to 1,300 base pairs in length were recovered by electroelution. A 20-ng portion of this cDNA was tailed with deoxyC residues[21], annealed with 100 ng of plasmid pBR322 (ref. 22) which had been tailed with deoxyG residues at the *Pst*I site, and used to transform *E. coli* K12 strain 294 (ref. 23). Approximately 1,000 tetracycline-resistant, ampicillin-sensitive transformants were obtained per ng of cDNA. A rapid plasmid isolation procedure[24] was used to prepare about 1 μg of plasmid DNA from each of 500 individual transformants. Each DNA sample was denatured and aliquots were applied to each of three nitrocellulose filters following a published procedure[25].

The amino acid sequences of several tryptic fragments of human LeIF have been determined (W. Levy, J. Shively and S. P., unpublished results). This information permitted the design of synthetic deoxyoligonucleotides potentially complementary to different regions of LeIF mRNA. The two tryptic peptides T-1, and T-13 were selected because they had amino acid sequences requiring the synthesis of only 12 and four undecamers, respectively, to account for all possible coding sequences (Fig. 1). Four sets of deoxyoligonucleotide probes were synthesized for each sequence, containing either three (T-1A, B, C, D) or one (T-13A, B, C, D) oligonucleotides each.

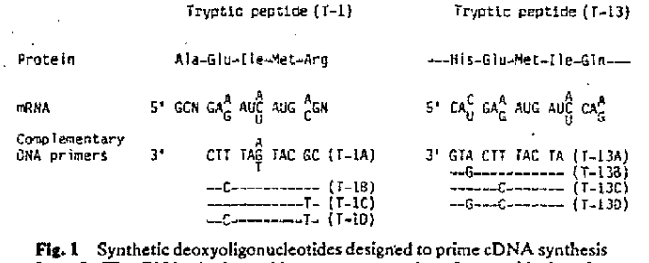

Fig. 1 Synthetic deoxyoligonucleotides designed to prime cDNA synthesis from LeIF mRNA. Amino acid sequences are given for peptide 1 and a portion of peptide 13 derived from a tryptic digest of human LeIF-β1 (W. Levy, J. Shively and S. P., unpublished results). All potential mRNA sequences coding for these peptides are shown. The indicated complementary deoxyoligonucleotides 11-bases long were chemically synthesized by the phosphotriester method[26]. Four individual primers were prepared in the T-13 series. The 12 T-1 primers were prepared in four pools of three primers each using a strategy described elsewhere[15]. ^{32}P-labelled cDNA was prepared from these primers using published reaction conditions[27]. The reactions (60 μl) were performed in 20mM Tris-HCl (pH 8.3), 20 mM KCl, 8 mM $MgCl_2$, 30 mM β-merceptoethanol. Reactions included one μg of each primer (that is 12 μg total for T-1 series, 4 μg total for T-13 series), two μg of 12S fraction mRNA from induced cells (or 10 μg of poly(A) mRNA from uninduced cells), 0.5 mM dATP, dGTP, dTTP, 200 μCi [α ^{32}P]dCTP (2–3,000 Ci mmol^{-1}; Amersham), and 60 U reverse transcriptase. Product was separated from unincorporated label by gel filtration on a 10-ml Sephadex G-50 column, treated with 0.3 M NaOH for 30 min at 70°C to destroy RNA, and neutralized with HCl. Hybridizations were performed as described[15,25].

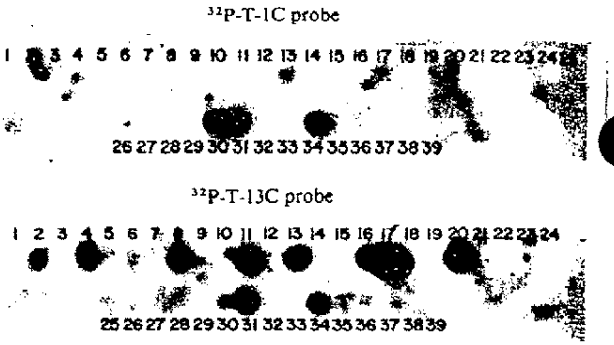

Fig. 2 Hybridization of potential LeIF cDNA recombinant plasmids with ^{32}P-labelled synthetic deoxyoligonucleotides. Plasmid DNA from the 39 clones was prepared by a standard cleared lysate procedure[38] and purified by Biorad Agarose A-50 column chromatography. Samples (3 μg) of each preparation were linearized by treatment with *Eco*RI, denatured in alkali and spotted on two separate nitrocellulose filters (1.5 μg per spot)[25]. Individual synthetic deoxyoligonucleotide primers and primer pools were phosphorylated with [γ ^{32}P]ATP as follows: 50 pmol of oligonucleotide and 100 pmol of [γ ^{32}P]ATP (2,500 Ci mmol^{-1}; New England Nuclear) were combined in 30 μl of 60 mM Tris-HCl (pH 8) 10 mM $MgCl_2$, 15 mM β-merceptothanol. Two units of T4 polynucleotide kinase were added and, after 30 min at 37°C, ^{32}P-labelled primers were purified by chromatography on 10-ml Sephadex G-50 columns. Hybridizations were performed using 10^6 c.p.m. of primer T-13C or 3×10^6 c.p.m. of primer pool T-1C. The hybridizations were performed at 15 °C for 14 h in 6×SSC, 10×Denhardt's solution, as described by Wallace *et al.* [28]. Filters were washed for 5 min (three times) at 0 °C in 6×SSC, dried, and exposed to X-ray film. Results are shown above for primer T-13C and primer pool T-1C.

These synthetic deoxyoligonucleotides were used to prime the synthesis of radiolabelled single-stranded cDNA for use as hybridization probes (see Fig. 1 legend). The template mRNA was either the 12S RNA from KG-1 cells induced with Sendai virus (8,000 U IFN activity μg^{-1}) or total poly(A) mRNA from uninduced leukocytes (<10 U IFN activity μg^{-1}).

The three sets of nitrocellulose filters containing the 500 plasmid samples were hybridized with three different cDNA probes: (1) induced cDNA primed with the T-1 set of primers, (2) T-13-primed induced cDNA, and (3) uninduced cDNA prepared by using both sets of primers. Clones were considered positive if they hybridized more strongly to one or both of the induced cDNA probes (1) and (2) than to the total uninduced probe(3). Thirty clones (pL1–pL30) were selected from the 500 for further analysis.

At this time a partial-length (~750-base pair) LeIF cDNA recombinant plasmid pIFL104, as identified by a mRNA hybridization selection procedure, became available[18]. A unique 260-base pair *Bgl*II restriction fragment isolated from this clone was labelled with ^{32}P (ref. 26) and used as probe to screen another 400 transformants by an *in situ* colony screening procedure[27]. Nine colonies (pL31–pL39) were identified which hybridized to different extents with this probe. Plasmid DNA was prepared from all 39 potential LeIF cDNA clones and rescreened with the same 260-base pair DNA probe using the dot hybridization procedure[25]. Three plasmids (pL4, pL31, pL34) exhibited very strong hybridization signals, four (pL13, pL30, pL32, pL36) hybridized moderately, and three (pL5, pL8, pL14) hybridized weakly with the probe.

The 39 potential LeIF cDNA recombinant plasmids were also screened with ^{32}P-labelled synthetic undecamers (individual T-1 primer pools or individual T-13 primers) directly as hybridization probes. The hybridization conditions were chosen such that perfect base pairing should be required for detectable hybridization signals[28]. Plasmid pIFL104 was found to give significant hybridization with primer pool T-1C and primer T-13C, but no detectable hybridization with the other undecamers. As shown in Fig. 2, several of the 39 potential LeIF plasmids (pL2, 4, 13, 17, 20, 30, 31, 34) also hybridized with both of these probes. Restriction analysis revealed that only one of these plasmids, pL31, also contained a 260-base pair internal *Bgl*II fragment.

Pst I digestion of pL31 showed the size of the cDNA insert to be approximately 1,000 base pairs.

DNA sequence of recombinant plasmid pL31

The entire *Pst*I insert of pL31 was sequenced by both the Maxam–Gilbert chemical method[29] and an enzymatic method[30] using the bacteriophage M13 cloning vector mp7 (P. S., unpublished results). The DNA sequence is shown in Fig. 3. Protein sequence information from LeIF (ref. 13; W. Levy, J. Shively and S. P., unpublished results) permitted the determination of the correct translational reading frame and allowed us to predict the entire LeIF type A (our term for the LeIF encoded by pL31) amino acid sequence, including a precursor segment or signal peptide. The first ATG translational initiation codon is found 60 nucleotides from the 5′ end of the sequence and is followed, 188 codons later, by a TGA termination triplet; there are 335 untranslated nucleotides at the 3′ end, followed by a poly(A) sequence. The putative signal peptide (presumably involved in the secretion of mature LeIF from leukocytes) is 23 amino acids long. The 165-amino acid polypeptide constituting the mature LeIF has a calculated molecular weight of 19,390. The DNA sequence also shows that the tryptic peptides T1 and T13 of LeIF β_1 (Fig. 1) correspond to amino acids 145–149 and 57–61, respectively, of LeIF A. The actual DNA coding sequences found in these two regions are those represented by primer pool T-1C and primer T-13C, as the hybridization data in Fig. 2 had suggested.

The amino acid sequence deduced from the DNA sequence of LeIF type A has only partial identity with the directly determined NH_2-terminal amino acid sequences of LeIFα1 (20 out of 22 positions)[13] and human lymphoblastoid IFN (16 out of 20 positions)[12]. It also differs significantly from the complete amino acid sequence recently deduced from the nucleotide sequence of LeIF I (ref. 31). LeIF I codes for a mature IFN of 166 amino acids, as compared to 165 amino acids for LeIF A. The extra amino acid (aspartic acid) of LeIF I is between amino acids 43 and 44 of LeIF A. Furthermore, only 137 of the remaining 165 amino acids (83%) are identical. The single longest stretch of homology between the two LeIFs is 19 amino acids (residues 132–150), and in one region (residues 100–106) only two out of seven amino acids are identical. The amino acid homology in the signal peptide sequence is 74% (17/23 identical residues). The glycine–cysteine cleavage site between the signal and IFN sequence is the same in both IFNs. In addition, the 3′ untranslated regions of LeIF A and LeIF I mRNAs (335 compared with 242 nucleotides) differ considerably. The explanation for these discrepancies lies in the fact that there is a family of non-identical LeIF genes (including pL4, pL13, pL30, pL32), evidence for which will be reported soon.

Expression of leukocyte pre-interferon A

The precursor form of LeIF A (Le-preIF A) was expressed in *E. coli* by ligating the 1,000-base pair *Pst*I insert into the *Pst*I site of the plasmid pNCV. pNCV is a derivative of pBR322 containing part of the *E. coli* tryptophan operon (extending from the promoter–operator region through the *trp* E gene, with a deletion[32] of a portion of the *trp* leader and *trp* E coding regions) which has been modified to contain a unique *Pst*I site at the C-terminal end of the *trp* E protein coding sequence (H. H., unpublished results). The LeIF A DNA sequence can be inserted into pNCV in either of two orientations. As shown in Table 1, only plasmids having inserts which are orientated correctly with respect to the direction of transcription from the *trp* promoter give detectable IFN activity. Extracts of *E. coli* containing the plasmid pLe-preIF A2 gave approximately 480,000 U of activity per litre of culture, considerably higher than the maximal 20,000 U l^{-1} reported by Nagata *et al.* for Le-preIF D (ref. 16). The Le-preIF A produced by *E. coli* can be visualized by staining and co-migrates with human growth hormone (191 amino acids; molecular weight 22,000) on SDS-polyacrylamide gels (J. Perry, unpublished results). This suggests that the expression of Le-preIF A in pNCV is due to translation initiating at the signal peptide ATG start codon.

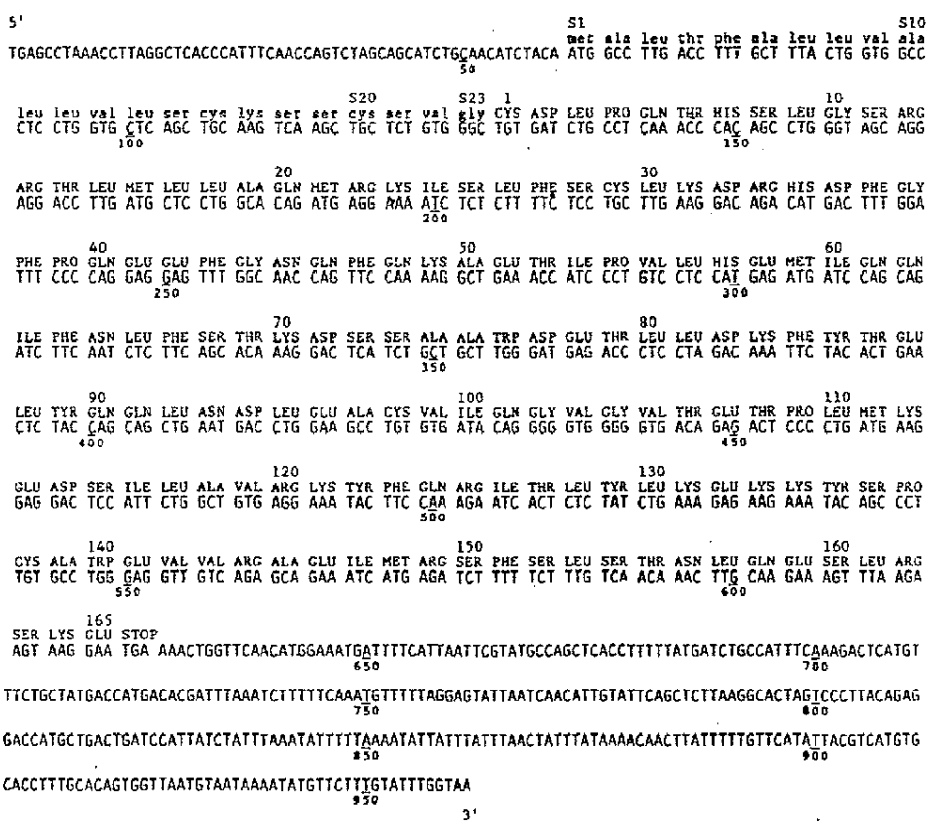

Fig. 3 Nucleotide and amino sequences of pL31 (LeIF A). The DNA sequences of both strands of the *Pst*I insert were determined by the Maxam–Gilbert technique and by the dideoxy chain termination procedure[30] after subcloning *Sau*3a fragments of pL31 into the M13 vector mp7 (P.S., unpublished results). Numbers above each line refer to amino acid position (S refers to signal peptide) and numbers below each line to nucleotide position.

Table 1 Interferon activity in extracts of *E. coli*

E. coli 294 transformed by:	Cell density (cells ml^{-1})	IFN activity (U per ml culture)	LeIF molecules per cell
pLe-preIF A2	3.5×10^8	480	120
pLe-preIF A3	3.5×10^8	0	0
pLeIF A25	3.5×10^8	36,000	9,000
pLeIF A25	1.8×10^9	250,000	12,000
pLeIF A118	3.5×10^8	20	5

The 1,000-base pair *Pst*I insert (0.5 μg) from pL31 was ligated to 0.2 μg of *Pst*I-cleaved pNCV and the product used to transform *E. coli* 294. Recombinant plasmids containing inserted LeIF cDNA were identified by colony hybridization[27] using the ^{32}P-labelled 260-base pair *Bgl*II fragment from pL31 as a hybridization probe. Digestion of these plasmids with *Pvu*II identified clones with the LeIF A DNA sequence orientated in the correct orientation (pLe-preIF A2) and in the opposite orientation (pLe-preIF A3). The construction of pLeIF A25 and pLeIF A118 for direct expression of LeIF A is described in Fig. 4 legend. pLeIF A118 has the *trp* promoter orientated such that transcription proceeds away from the LeIF A gene. Extracts were prepared for IFN assay as follows. One-ml cultures were grown in L-broth containing 5 μg ml^{-1} tetracycline to an A_{550} of ~1.0, then diluted into 25 ml of M9 medium containing 0.2% glucose, 0.5% casamino acids and 5 μg ml^{-1} tetracycline. 10-ml samples were collected by centrifugation when A_{550} reached 1.0 and cell pellets were suspended in 1 ml of 15% sucrose, 50 mM Tris-HCl (*p*H 8.0), 50 mM EDTA. One mg of lysozyme was added and, after 5 min at 0 °C, cells were disrupted by sonication. The samples were centrifuged for 10 min at 15,000 r.p.m. in a Sorvall SM-24 rotor. IFN activity in the supernatants was determined by comparison with LeIF standards by the cytopathic effect (CPE) inhibition assay[2]. To determine the number of IFN molecules per cell, a LeIF specific activity of 4×10^8 U mg^{-1} was used[9]. To measure *p*H2 stability, the 250,000 U ml^{-1} extract of *E. coli* 294/pLeIF A25 was diluted 500-fold with MEM giving a concentration of 500 U ml^{-1}. A LIF standard (Wadley Institute) previously titrated against the NIH LIF standard, was also diluted to a final concentration of 500 U ml^{-1}. Aliquots (1 ml) were adjusted to *p*H 2 with 1 M HCl, incubated at 4 °C for 52 h, neutralized by addition of NaOH and IFN activity determined by the standard CPE inhibition assay[2]. To measure neutralization by antibody, 25-μl aliquots of the 500 U ml^{-1} samples (untreated) were incubated with 25 μl of rabbit anti-human LeIF (10,000 neutralizing units ml^{-1}) for 60 min at 37 °C, centrifuged at 12,000*g* for 5 min and the supernatant assayed.

Direct expression of mature leukocyte interferon

The procedure followed to express LeIF A directly as a mature IFN polypeptide was a variation of that employed previously for human growth hormone[20]. As shown in Fig. 4, a *Sau*3a restriction endonuclease site is conveniently located between codons 1 and 2 of the LeIF A sequence. Two synthetic deoxyoligonucleotides were designed which incorporate an ATG translational initiation codon, restore the codon for amino acid 1 (cysteine), and create an *Eco*RI sticky end. These oligomers were ligated to a 34-base pair, *Sau*3a–*Ava*II fragment of pL31. The resulting 45-base pair product was ligated to two additional DNA fragments to construct an 865-base pair synthetic–natural hybrid gene which codes for LeIF A and which is bounded by *Eco*RI and *Pst*I restriction sites. This gene was inserted into pBR322 between the *Eco*RI and *Pst*I sites to give the plasmid pLeIF A1.

Next, a 300-base pair *Eco*RI fragment was constructed which contains the *E. coli trp* promoter, operator, and the *trp* leader ribosome binding site but stops short of the ATG sequence for initiation of translation of the leader peptide (D. Kleid and D. Yansura, unpublished results). This DNA fragment was cloned into the *Eco*RI site of pLeIF A1. Transformants carrying plasmids with the *trp* promoter orientated in either possible orientation were assayed for IFN activity (Table 1). Clone pLeIF A25, in which the *trp* promoter was inserted in the desired orientation, gives high levels of activity (2.5×10^8 U l^{-1}). Low levels of activity are obtained even if the *trp* promoter reads away from the LeIF A gene, as in the case of pLeIF A118 (2.0×10^4 U l^{-1}). The IFN produced by *E. coli* 294/pLeIF A25 behaves like authentic human LeIF; it is stable to treatment at *p*H2 and is

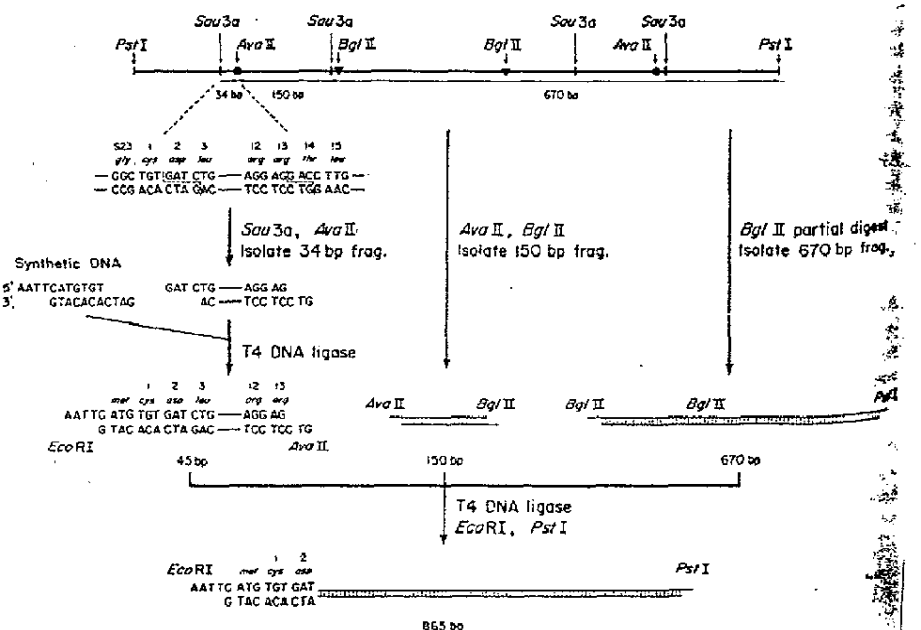

Fig. 4 Construction of a gene coding for the direct synthesis of mature human LeIF A in *E. coli*. 250 μg of plasmid pL31 were digested with *Pst*I and the 1,000-base pair insert isolated by gel electrophoresis on a 6% polyacrylamide gel. Approximately 40 μg of insert were electroeluted from the gel and divided into three aliquots for further digestion. *a*, A 16-μg sample of this fragment was partially digested with 40 U of *Bgl*II for 45 min at 37 °C and the reaction mixture purified on a 6% polyacrylamide gel. Approximately 2 μg of the desired 670-base pair fragment were recovered. *b*, Another sample (8 μg) of the 1,000-base pair *Pst*I insert was restricted with *Ava*II and *Bgl*II. One μg of the indicated 150-base pair fragment was recovered after gel electrophoresis. *c*, 16 μg of the 1,000-base pair piece were treated with *Sau*3a and *Ava*II. After electrophoresis on a 10% polyacrylamide gel, approximately 0.25 μg (~10 pmol) of the 34-base pair fragment were recovered. The two indicated deoxyoligonucleotides, 5′-dAATTCATGTGT (fragment 1) and 5′-dGATCACACATG (fragment 2) were synthesized by the phosphotriester procedure[36]. Fragment 2 was phosphorylated as follows: 200 μl (~40 pmol) of [γ^{32}P]ATP (5,000 Ci $mmol^{-1}$; Amersham) was dried down and resuspended in 30 μl of 60 mM Tris-HCl (*p*H 8), 10 mM $MgCl_2$, 15 mM β-mercaptoethanol, containing 100 pmol of DNA fragment and 2 U of T4 polynucleotide kinase. After 15 min at 37 °C, 1 μl of 10 mM ATP was added and the reaction allowed to proceed another 15 min. The mixture was then heated at 70 °C for 15 min, combined with 100 pmol of 5′-OH fragment 1 and 10 pmol of the 34-base pair *Sau*3a–*Ava*II fragment. Ligation was performed for 5 h at 4 °C in 50 μl of 20 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM ATP and 10 U T4 DNA ligase. The mixture was electrophoresed on a 6% PA gel and the 45-base pair product recovered by electroelution. 860,000 Cerenkov c.p.m. were recovered (~30 ng, 1 pmol), combined with 0.5 μg (5 pmol) of the 150-base pair *Ava*II–*Bgl*II fragment and 1 μg (2 pmol) of the 670-base pair *Bgl*II–*Pst*I fragment. The ligation was performed at 20 °C for 16 h using 20 U of T4 DNA ligase. The ligase was inactivated by heating to 65 °C for 10 min. The mixture was then digested with *Eco*RI and *Pst*I to eliminate polymers of the gene. The mixture was purified by 6% polyacrylamide gel electrophoresis. 36,000 c.p.m. (~0.04 pmol, 20 ng) of 865-base pair product were isolated. One half (10 ng) of this was ligated into pBR322 (0.3 μg) between the *Eco*RI and *Pst*I sites. Transformation of *E. coli* 294 gave rise to 70 tetracycline-resistant, ampicillin-sensitive transformants. Plasmid DNA isolated from 18 of these transformants was digested with *Eco*RI and *Pst*I. 16 of the 18 plasmids had an *Eco*RI–*Pst*I fragment 865 base pairs in length. One μg of one of these, pLeIF A1, was digested with *Eco*RI and ligated to a 300-base pair *Eco*RI fragment (0.1 μg) containing the *E. coli trp* promoter and *trp* leader ribosome binding site. Transformants containing the *trp* promoter were identified using a ^{32}P-*trp* probe in conjuction with the Grunstein–Hogness colony screening procedure[37]. An asymmetrically located *Xba*I site in the *trp* fragment allowed us to determine recombinants in which the *trp* promoter was orientated in the direction of the LeIF A gene. bp, Base pair.

Table 2 Antiviral effect of various LeIF preparations against EMC virus infection of squirrel monkeys

Treatment	Survivors	Serum PFU ml^{-1} Day 2	Day 3	Day 4
Control		10^3	3×10^4	$>10^5$
Bacterial proteins)	0/3	0 } 3	0 } 10^4	1,200 } $>3.4\times10^4$
		0	0	0
Bacterial LeIF A		0	0	0
	3/3	0	0	0
		0	0	0
LeIF standard		0	0	0
	3/3	0	0	0
		0	0	0

All monkeys were male (average weight 713 g) and had no EMC virus antibodies prior to infection. The monkeys were infected intramuscularly with $100\times LD_{50}$ EMC virus (determined in mice). The control treated monkeys died at 134, 138 and 164 h post-infection. IFN treatments of 10^6 U were intravenously at −4, +4, 23, 29, 48, 72, 168 and 240 h, relative to infection. The bacterial LeIF was a column chromatography fraction (R. Wetzel, unpublished results) from a lysate of *E. coli* 294/pLeIF A25 at a specific activity of 7.4×10^6 U mg^{-1} protein. The control bacterial proteins were an equivalent column fraction from a lysate of *E. coli* 294/pBR322 at twice the total protein concentration. The LeIF standard was Sendai virus-induced IFN from normal human buffy-coat cells, purified chromatographically to a specific activity of 32×10^6 U per mg protein. Survival of the monkeys in the IFN-treated group is significant at the 5% level by a log rank χ^2 analysis[39]. PFU, plaque forming unit.

neutralized by rabbit anti-human LIF antibodies. The IFN has been partially purified and has an apparent molecular weight of 20,000 (R. Wetzel and M. Ross, unpublished results).

In vivo antiviral activity of LeIF A

The *in vivo* efficacy of IFN needs the presence of macrophages and NK cells, and the *in vivo* mode of action seems to involve stimulation of these cells[33–35]. Thus, it remained possible that the IFN produced by *E. coli* 294/pLeIF A25, though having antiviral activity in the cell culture assay, would not be active in infected animals. Moreover, the *in vivo* antiviral activity of the bacterially produced, non-glycosylated LeIF A might be different from the IFN derived from human 'buffy coat' leukocytes. Therefore the biological activity of bacterially synthesized LeIF A (~2% pure) was compared with buffy coat LeIF (~8% pure) in lethal encephalomyocarditis (EMC) virus infection of squirrel monkeys (Table 2). The control monkeys showed progressive lethargy, loss of balance, flaccid paralysis of the hind limbs and watering of the eyes commencing ~8 h before death. The IFN-treated monkeys showed none of these signs; they remained active at all times and developed no viremia (Table 2). The one monkey in the control group which did not develop viremia by 4 days died latest (164 h post-infection) but showed high titres of virus in the heart and brain on post mortem. The IFN-treated monkeys did not develop antibodies to EMC virus as determined 14 and 21 days after infection. These results demonstrate that the antiviral effects of LeIF preparations in the infected animals can be attributed solely to IFN because the contaminating proteins are quite different in the bacterial and buffy coat preparations. In addition, these results indicate that glycosylation is not required for the *in vivo* antiviral activity of LeIF A. The pharmacokinetics and toxic effects of the natural and bacterially produced LeIFs are currently being compared.

Discussion

With 12S poly(A) mRNA from induced human leukocytes, a full length LeIF A cDNA has been synthesized and cloned in the *E. coli* vector pBR322. This particular clone (pL31) was identified by colony screening using a DNA fragment from a shorter LeIF cDNA clone[18] as hybridization probe. pL31 was also found to hybridize to synthetic deoxyoligonucleotide probes whose sequences were based upon LeIF amino acid sequence data. Several additional types of LeIF recombinant plasmids were identified by hybridization to radiolabelled LeIF cDNA probes. These probes were prepared by using the synthetic deoxyoligonucleotides as primers for the synthesis of cDNA using 12S poly(A) mRNA from induced cells as template. This approach should be generally applicable for the identification of recombinant plasmids obtained through cDNA cloning of rare mRNA species, assuming that some protein sequence information is available.

The 961-base pair DNA sequence of pL31 codes for 188 amino acids, 23 of which are probably cleaved during the process of secretion of mature LeIF from leukocytes. The 188-amino acid leukocyte pre-IF A was expressed in *E. coli* under *trp* promoter control and bacterial extracts were found to be biologically active in tissue culture assay. It is not yet known if the IFN activity is associated with the pre-interferon itself, or if a small proportion of Le-preIF A is processed by *E. coli* to a biologically active form. A plasmid was also constructed which codes for high level expression of the 165 amino acid LeIF A preceded only by a methionine. The present yield of 2.5×10^8 U per litre of culture corresponds to about 600 μg of IFN per litre of culture, assuming an LeIF specific activity of 4×10^8 U mg^{-1} (ref. 9).

This bacterially synthesized LeIF A exhibits the *p*H 2 stability and immunological characteristics of authentic human LeIF, and it protects squirrel monkeys against lethal infection with EMC virus when treatments are initiated prior to viral challenge. Studies are under way to determine if bacterially produced LeIF A has therapeutic, as well as prophylactic, value. Additionally, the *in vivo* activity of LeIF A is being compared with the activities of bacterially produced human fibroblast IFN[15], and several other types of bacterially expressed LeIF.

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1. Isaacs, A. & Lindenmann, J. *Proc. R. Soc.* B147, 258–267 (1957).
2. Stewart, W. E. II *The Interferon System* (Springer, New York, 1979).
3. Havell, E. A. *et al. Proc. natn. Acad. Sci. U.S.A.* 74, 3287–3291 (1977).
4. Cavalieri, R. L., Havell, E. A., Vilcek, J. & Pestka, S. *Proc. natn. Acad. Sci. U.S.A.* 74, 3287–3291 (1977).
5. Gresser, I., Bandu, M. T., Brouty-Boye, D. & Tovey, M. *Nature* 251, 543–545 (1974).
6. Pestka, S., McInnes, J., Havell, E. & Vilcek, J. *Proc. natn. Acad. Sci. U.S.A.* 72, 3898–3901 (1975).
7. Reynolds, F. H. Jr, Premkumar, E. & Pitha, P. M. *Proc. natn. Acad. Sci. U.S.A.* 72, 4881–4885 (1975).
8. Thang, M. N., Thang, D. C., De Maeyer, E. & Montagnier, L. *Proc. natn. Acad. Sci. U.S.A.* 72, 3975–3977 (1975).
9. Rubinstein, M. *et al. Proc. natn. Acad. Sci. U.S.A.* 76, 640–644 (1979).
10. Zoon, K. C., Smith, M. E., Bridgen, P. J., zur Nedden, D. & Anfinsen, C. B. *Proc. natn. Acad. Sci. U.S.A.* 76, 5601–5605 (1979).
11. Knight, E., Jr, Hunkapillar, M. W., Korant, B. D., Hardy, R. W. F. & Hood, L. E. *Science* 207, 525–526 (1980).
12. Zoon, K. C. *et al. Science* 207, 527–528 (1980).
13. Levy, W. P., Shively, J., Rubinstein, M., Del Valle, U. & Pestka, S. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
14. Taniguchi, T. *et al. Proc. Japan Acad.* 55B, 464–469 (1979).
15. Goeddel, D. V. *et al. Nucleic Acids Res.* (in the press).
16. Nagata, S. *et al. Nature* 284, 316–320 (1980).
17. Koeffler, H. P. & Golde, D. W. *Science* 200, 1153–1154 (1978).
18. Maeda, S. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
19. Wickens, M. P., Buell, G. N. & Schimke, R. T. *J. biol. Chem.* 253, 2483–2495 (1978).
20. Goeddel, D. V. *et al. Nature* 281, 544–548 (1979).
21. Chang, A. C. Y. *et al. Nature* 275, 617–624 (1978).
22. Bolivar, F. *et al. Gene* 2, 95–113 (1977).
23. Backman, K., Ptashne, M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* 73, 4174–4178 (1976).

24. Birnboim, H. C. & Doly, J. *Nucleic Acids Res.* **7**, 1513–1523 (1979).
25. Kafatos, F. C., Jones, C. W. & Efstratiadis, A. *Nucleic Acids Res.* **7**, 1541–1552 (1979).
26. Taylor, J. M., Illemensee, R. & Summers, S. *Biochim. biophys. Acta* **442**, 324–330 (1976).
27. Grunstein, M. & Hogness, D. S., *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965 (1975).
28. Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J. & Itakura, K. *Nucleic Acids Res.* **6**, 3543–3557 (1979).
29. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
30. Smith, A. J. H. *Meth. Enzym.* **65**, 560–580 (1980).
31. Mantei, N. *et al. Gene* **10**, 1–10 (1980).
32. Miozzari, G. F. & Yanofsky, C. *J. Bact.* **133**, 1457–1466 (1978).
33. Herberman, R. B. *et al. Cancer Treat. Rep.* **62**, 1893–1896 (1978).
34. Gidlund, M., Orn, A., Wigzell, H., Senik, A. & Gressor, I. *Nature* **273**, 759–761 (1978).
35. Stebbing, N., Dawson, K. M. & Lindley, I. J. D. *Infect. Immun.* **19**, 5–11 (1978).
36. Crea, R., Kraszewski, A., Hirose, T. & Itakura, K. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5765–5769 (1978).
37. Noyes, B., Mevarech, M., Stein, R. & Agarwal, K. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1770–1774 (1979).
38. Clewell, D. B. & Helinski, D. R. *Biochemistry* **9**, 4428–4440 (1970).
39. Peto, R. & Pike, M. C. *Biometrics* **29**, 579–584 (1973).